Phytopathol. Mediterr. (2009) 48, 150–154

Conidia dispersal of *Diplodia* species in a French vineyard

PHILIPPE KUNTZMANN[1], STÉPHANIE VILLAUME[2] and CHRISTOPHE BERTSCH[3]

[1]Institut Français de la Vigne et du Vin (ENTAV-ITV France) Station Alsace, France
[2]Conseil Interprofessionnel des Vins d'Alsace, Maison des Vins d'Alsace, Colmar, France
[3]Laboratoire Vigne Biotechnologie et Environnement, Université de Haute-Alsace, Colmar France

Summary. Diseases caused by species of Botryosphaeriaceae lead to significant losses of grape yield. Species in this family produce foliar symptoms similar to, but distinguishable from esca, and the diseases they cause are generally named black dead arm (BDA). Botryosphaeriaceae species are ascomycetes frequently isolated from grapevine stocks showing decline or dieback symptoms. It is therefore useful to know what is the spore dissemination period of Botryosphaeriaceae in the vineyard. The objective of this study was to determine the peak periods of conidial release by some *Diplodia* spp. in the Botryosphaeriaceae in grapevines and to ascertain the climatic factors that influence inoculum availability and dispersal. Spore dispersal from *Vitis vinifera* was studied from 2005 to 2006 in a French vineyard. Spores of *Diplodia seriata* and *D. mutila* were trapped throughout the year. Spore release from *D. seriata* peaked during the vegetative growth period, while *D. mutila* released its spores later.

Key words: grapevine, Botryosphaeriaceae spp., conidia dispersal, black dead arm

Introduction

The Botryosphaeriaceae represent a diverse family including more than 2000 taxa (index fungorum; http://www.indexfungorum.org). Diseases caused by Botryosphaeriaceae species result in significant losses to a variety of economically valuable agricultural crops, including grapevines. Many species of Botryosphaeriaceae cause disease symptoms such as dieback and cankers on numerous woody and non-woody hosts (Eldridge, 1961; Buchanan, 1967; Punithalingam, 1970). The species are saprophytic, parasitic or endophytic on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts (Barr, 1972). Diseases caused by the Botryosphaeriaceae mostly follow the onset of stress from factors other than the Botryosphaeriaceae infection itself (Blodgett and Stanosz, 1997; Schoeneweiss, 1981; Swart and Wingfield, 1991). The taxonomy of Botryosphaeriaceae species is based on anamorph morphology, which is the most frequently encountered in nature (Crous *et al*. 2006).

Various *Botryosphaeria* species have been reported in grapevines. *Diplodia mutila* Fr., (teleomorph "*Botryosphaeria*" *stevensii* Shoemaker) was shown to cause BDA in grapevines (Lehoczky, 1974). *D. seriata* De Not. (teleomorph "*Botryosphaeria*" *obtusa* (Schw.) Shoemaker) has been associated with symptoms of BDA in both Italian (Cristinzio, 1978; Rovesti and Montermini, 1987) and French vineyards (Larignon and Dubos, 2001; Larignon *et al*., 2001). *Botryosphaeria dothidea* (as *B. ribis* Gross. & Duggar) is reported to cause the same symptoms, and furthermore, dark lesions developed on artificially inoculated one-year-old canes (Larignon and Dubos, 2001). Field observations have shown that BDA produces a yellow pigmentation of the leaves in white grape varieties, and a red pigmentation of the leaves in red grape varieties. Brown streak-

Corresponding author: P. Kuntzmann
Fax: +33 (0)3 89224965
E-mail: philippe.kuntzmann@vignevin.com

ing of the wood under the bark was also observed (Larignon and Dubos, 2001).

The objective of this study was to determine the peak periods of conidial release by Botryosphaeriaceae (especially *D. seriata* and *D. mutila*) in a vineyard, and to ascertain the weather factors that influence inoculum availability and dispersal.

Materials and methods

Vineyard samples

The experimental vineyard was located in the Alsace grape-growing region in the east of France and was planted in 1987 with *Vitis vinifera* cv. Gewurztraminer. Grapevine plants were grafted on 161-49 C rootstock. The vines were trained in "double Guyot". This vineyard has never been sprayed with sodium arsenite, so we can ignore any effect such a treatment could conceivably have had on the *Diplodia*.

Spore trapping

Conidia were trapped from 6 April 2005 to 31 December 2006 on seventeen microscope glass slides coated with petroleum jelly and placed at various locations in the vineyard. The slides were placed on thirteen grapevine trunks that had shown symptoms of BDA in previous years, at sites such as old pruning wounds or cankers, where any pycnidia of Botryosphaeriaceae could be visible. Eight slides were placed at the upper part of the trunk, eight on the middle of the trunk and one at the base of the trunk on the rootstock. The slides were placed as close as possible to the pycnidia, in a way designed to trap the spores but without hindering any rain falling on the pycnidia. The angle at which the slides were positioned relative to the trunks varied between slides, depending on the location of the pycnidia and the shape of the trunk.

Environmental monitoring

Temperature, relative humidity, and the occurrence of fog and snow were recorded daily by Météo-France (Domaine du Hohrain No. 68287003, alt. 285m, lat. 47°57'9N, long. 07°17'2E).

Notation and expression of the results

Spores were counted every 7 days from 6 April to 21 December 2005 and from 5 April to 26 September 2006, and every 14 days from 5 January to 29 March 2006 and 10 October to 31 December 2006. Spores were extracted by dissolving the petroleum jelly in 3 ml of hot (70°C) water. After homogenization, the spores were counted with a haemacytometer. Species were identified using the keys presented by Phillips (http://www.crem.fct.unl.pt/botryosphaeria_site/). The final count is expressed as the number of spores present on whole slides for each sampling date, calculated on the amount of water used to dissolve the jelly and the concentration given by the hemacytometer.

Results

Analysis of the results must take into account how long the slides were exposed in the field during the monitoring period. Thus, when considering two different periods in the reading frequency - for example winter 2006 versus summer 2006 - we can in this case only compare the overall global amount of spores trapped.

In 2005 and 2006, we identified *D. mutila* and *D. seriata* (Fig. 1–2). "*Botryosphaeria*" *quercuum* and *Neofusicoccum parvum* were detected in very small quantities (data not shown).

During 2005, 90% of the spores of *D. seriata* were trapped during the vegetative growth period (Fig. 1A). Rain episodes during spring and autumn seemed to be as important as summer rain in releasing Botryosphaeriaceae spores. Fog did not increase spore release (12 to 18 October 2005). At the sampling date of 30 November 2005, just after a snowfall some days before, spore release was quite low. But at the sampling date of 7 December 2005, which had been preceded by a rise in temperature (max 9°C) accompanied by rains, the release of spores was quite large.

As with *D. seriata*, 90% of *D. mutila* spores were released during the vegetative growth period. However, the release of *Diplodia mutila* spores started later than that of *D. seriata* spores (Fig. 1A). In 2005, more than 50% of *D. mutila* spores were released between August and November. After the fog episode from 12 to 18 October 2005 there was an increased emission of spores. When the sampling dates 30 November and 7 December 2005 were compared, the difference between the two *D. mutila* spore counts was smaller than between the two *D. seriata* spore counts.

In 2006, the number of trapped spores overall was smaller than in 2005 (Fig. 1B). *D. seriata* spores were produced throughout the year during rain episodes with temperatures of 9°C, with a peak between April and October 2006, corresponding to the vegetative growth period.

Spores of *D. mutila*, were also trapped during the vegetative growth period with a later release confirmed in 2006 (Fig. 1B). Dispersal of conidia was lower throughout the year, but with fewer peaks of release and a higher intensity 2 to 3 times in that year.

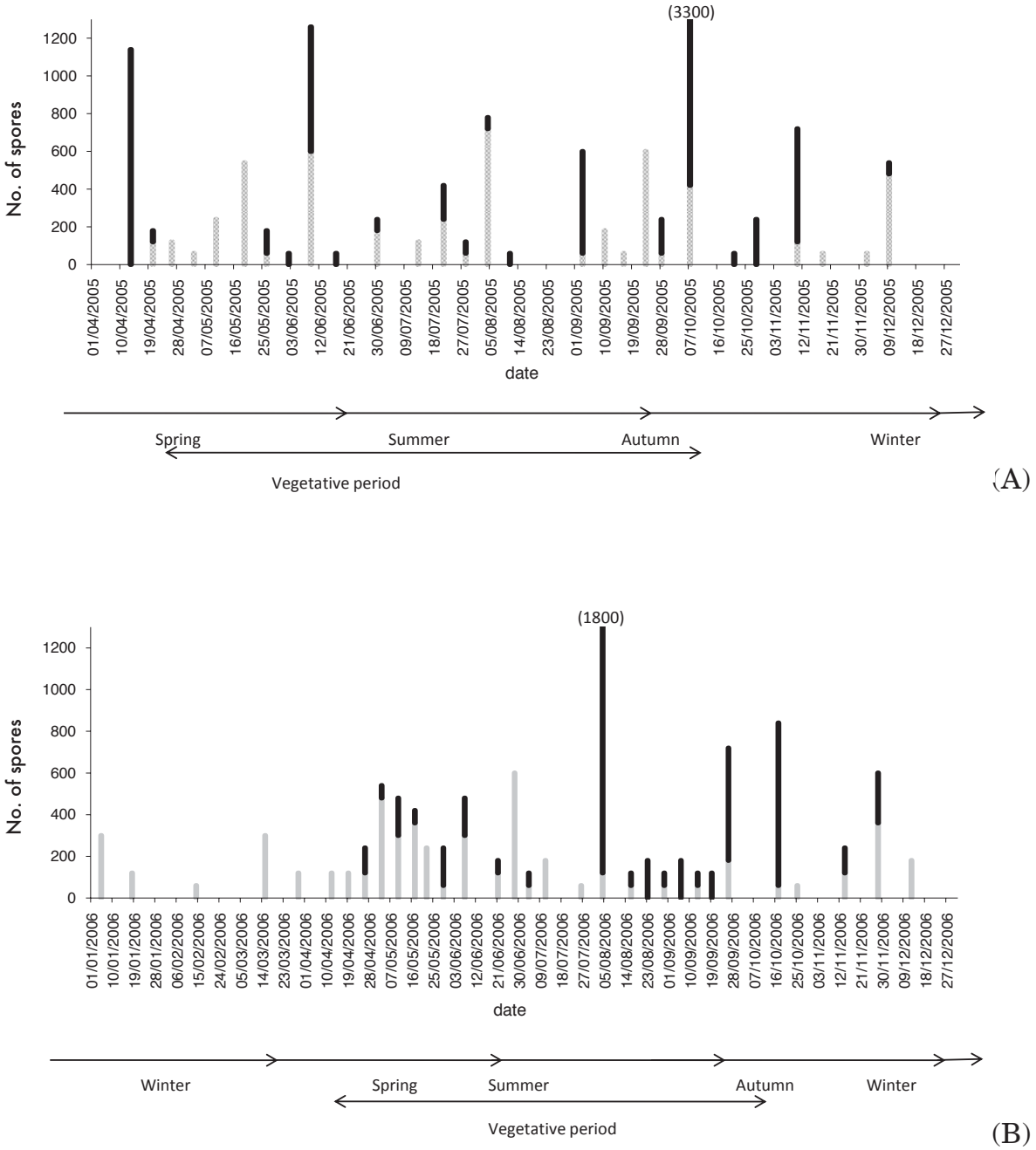
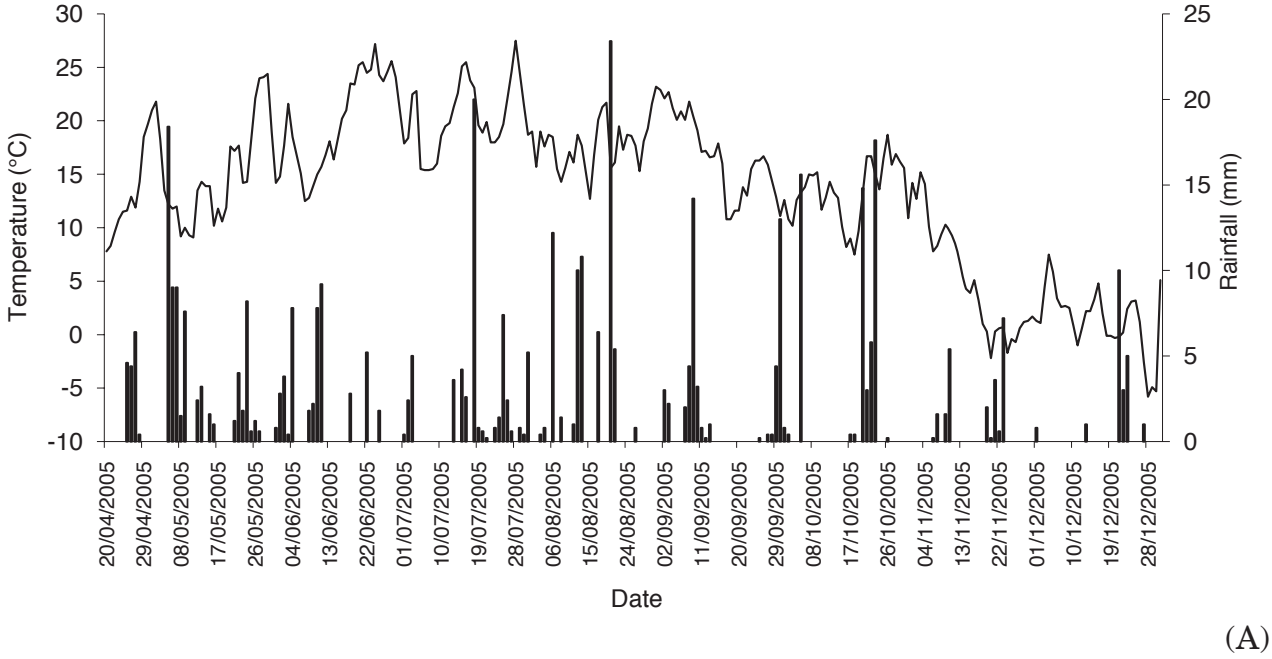
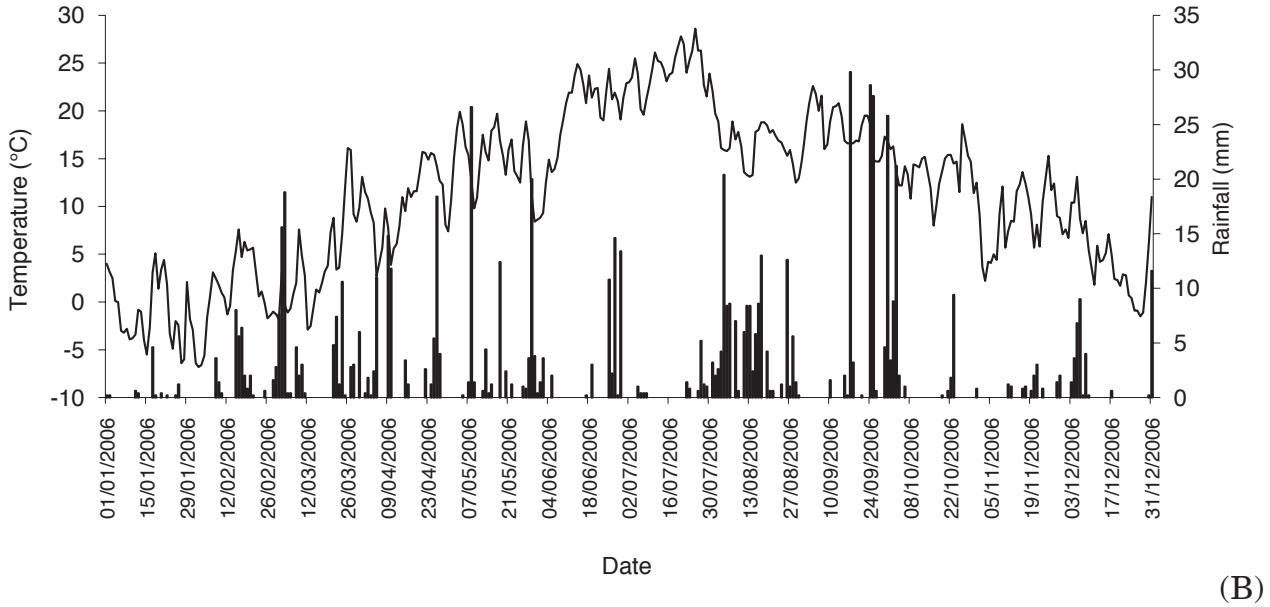


Fig 1. Cumulative quantity of conidia of *Diplodia seriata* (grey) and *Diplodia mutila* (black) trapped between 6 April 2005 and 21 December 2005 (A), and between 5 January 2006 and 31 December 2006 (B).



(A)



(B)

Fig 2. Temperature (line) and rainfall (columns) between 1 April and 31 December 2005 (A), and between 1 January and 31 December 2006 (B).

Discussion

Conidia of Botryopshaeriaceae could be a major cause of primary infections. Conidia of *D. seriata*, *D. mutila* and less frequently spores of *N. parvum* and *"Botryosphaeria" quercuum* were trapped during the study (2005–2006). Peak release occurred during the vegetative growth period. *Diplodia mutila* released its spores later than *D. seriata*, suggesting either that these fungi differ in their capacity to grow and sporulate, or that they merely differ in their response to weather factors. Particularly in the case of *D. mutila*, rain episodes in the inter-seasons led to a larger release of spores at the sampling dates, than during the summer, despite lower levels of rainfall. In the same way, fewer spores were trapped in 2006 than in 2005, even though 2006 was warmer and wetter than 2005. Copes (2003) showed that conidia production *in vitro* of *B. dothidea*, *D. seriata*, and *Lasiodiplodia theobromae* occured at 6°C. In the present study *D. seriata* conidia were trapped at 9°C. This finding and those of the present study underline that conidial production and sporulation could be initiated even during both mild and wet spells in Winter. However in the study we encountered some particularly cold and dry conditions in December 2005, January and February 2006, to judge by the low spore release we found in the field.

The study gives some indications on spore availability of some Botryosphaeriaceae in a cool temperate viticultural area. The spores were released throughout the year. The next step would be to determine the infection ways of the fungi, both during the vegetative growth season and at other times.

Acknowledgements

This study received financial support from VINIFLHOR and CASDAR.

Literature cited

Barr M.E. 1972. Preliminary studies on the *Dothideales* in temperate North America. *Contributions to the University of Michigan Herbarium* 9, 523–638

Blodgett T. and G.R. Stanosz, 1997. *Sphaeropsis sapinea* and host water stress in a red pine plantation in central Wisconsin. *Phytopathology* 87, 429–434.

Buchanan T.S., 1967. *Diplodia* twig blight of pine, important forest insects and diseases of mutual concern to Canada, United States and Mexico. North American Forestry Commission, Canadian Department of Forestry, Ottawa, Canada 189–191.

Copes W.E., 2003. Effect of temperature on sporulation of *Botryosphaeria dothidea*, *B. obtusa*, and *B. rhodina*. *Plant Disease* 88, 292–296.

Cristinzio G., 1978. Gravi attacchi di *Botryosphaeria obtusa* su vite in provincia di Isernia. *Informatore Fitopatologico* 6, 21–23.

Crous P.W., B. Slippers, M.J. Wingfield, J. Reeder, W.F.O. Marasas, A.J.L. Phillips, A. Alves, T. Burgess, P. Barber and J.Z. Groenewald, 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55, 235–253.

Eldridge K.G., 1961. Significance of *Diplodia pinea* in plantations. *Review of Applied Mycology* 41, 339.

Larignon P., and B. Dubos, 2001. Le black dead arm. Maladie nouvelle à ne pas confondre avec l'esca. *Phytoma* 538, 26–29.

Larignon P., R. Fulchic , L. Cere and B. Dubos. 2001. Observation on black dead arm in French vineyards. *Phytopathologia Mediterranea* 40, 336–342.

Lehoczky J., 1974. Black dead arm disease of grapevines caused by *Botryosphaeria stevensii* infection. *Acta Phytopathologica Academiae Scientarum Hungaricae* 9, 319–327.

Rovesti L. and A. Montermini, 1987. Un deperimento della vite causato da *Sphaeropsis malorum* diffuso in provincia di Reggio Emilia. *Informatore Fitopatologico* 1, 59–61.

Punithalingam, E. and J.M. Waterston, 1970. *Diplodia pinea*. *CMI Descriptions of pathogenic fungi and bacteria*, Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England, 273.

Schoeneweiss D.F. 1981. The role of environmental stress in diseases of woody plants, *Plant Disease* 65, 308–314.

Swart W.J. and Wingfield M.J., 1991. Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Disease* 75, 761–766.

Accepted for publication: March 17, 2009